

**A CORRELATIVE STUDY ON BRONCHO ALVEOLAR  
LAVAGE CYTOLOGY WITH HISTOPATHOLOGY IN  
PATIENTS WITH PULMONARY LESIONS**

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## **CERTIFICATE**

This is to certify that the dissertation entitled “**A CORRELATIVE STUDY ON BRONCHO ALVEOLAR CYTOLOGY WITH HISTOPATHOLOGY IN PATIENTS WITH PULMONARY LESIONS**” presented herewith by Dr. A.G. Krishnaveni to the faculty of pathology. The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D. Degree in pathology is a bonafide work carried out by her during the period July 2006 to June 2008 under my direct supervision and guidance.

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## **CONTENTS**

<b>Chapter</b>	<b>Title</b>	<b>Page No</b>
<b>1.</b>	<b>INTRODUCTION</b>	<b>1</b>
<b>2.</b>	<b>AIM OF STUDY</b>	<b>3</b>
<b>3.</b>	<b>REVIEW OF LITERATURE</b>	<b>4</b>
<b>4.</b>	<b>MATERIAL AND METHODS</b>	<b>45</b>
<b>5.</b>	<b>OBSERVATION AND RESULTS</b>	<b>48</b>
<b>6.</b>	<b>DISCUSSION</b>	<b>57</b>
<b>7.</b>	<b>SUMMARY AND CONCLUSION</b>	<b>62</b>
	<b>ANNEXURE - I (PROFORMA)</b>	
	<b>ANNEXURE - II (BAL TECHNIQUE)</b>	
	<b>ANNEXURE - III (STAINING TECHNIQUES)</b>	
	<b>ANNEXURE - IV (BIBLIOGRAPHY)</b>	
	<b>ANNEXURE - V (MASTER CHART)</b>	

## INTRODUCTION

The respiratory tract serves the dual purpose of supplying oxygen to and removing carbon dioxide from the circulating blood and is likely to develop all neoplastic and non-neoplastic diseases. Patients with diseases of the respiratory system generally present because of symptoms, an abnormality on a chest radiograph or both.

The diagnostic modalities available for assessing the patient with suspected or known respiratory system disease include imaging studies and techniques for acquiring biopsy specimens, some of which involve direct visualisation of part of respiratory system.

Bronchoscopy is one among such procedures which not only visualizes the respiratory system but also aids in obtaining the representative sampling material from the regions that are directly visualised and also from the more distal pulmonary parenchyma.

BAL is one among several techniques which provides sequential access to well preserved cells to study the natural history of the disease process.

As an investigative tool BAL has enormous potential. BAL has been found useful in diagnosing opportunistic infections in the lung, bronchoalveolar hemorrhage and alveolar proteinosis.

BAL has also been used to investigate the pathogenesis of such diverse lung conditions such as emphysema, ARDS, occupation lung disease, drug hypersensitivity reactions and asthma.

The cytological examination of cells obtained by BAL has been useful in the diagnosis of primary bronchogenic cancer and the metastatic cancer to the lung in particular lymphangitic carcinomatosis.

This study aims at studying the bronchoalveolar lavage samples obtained from the lungs in various pulmonary lesions and its histopathological correlation.

## **AIM OF STUDY**

1. To evaluate the broncho alveolar lavage sample of patients presented with respiratory symptoms such as cough, fever, hemoptysis, breathlessness and weight loss.
2. To study the abnormal smears and correlate the cytological finding with histopathological diagnosis
3. To assess the usefulness of the cytological study in the diagnosis of pulmonary lesions.

## **HISTORICAL ASPECTS**

Rigid bronchoscope was introduced in 1895 by Gustav Killian (1860-1921) in Germany. In 1897, Killian demonstrated first clinical application bronchoscope when he removed the piece of bone from right bronchus. He actually used rigid esophagoscope and a long forceps to do that. He is considered to be the “Father of bronchoscope”.

In 1904, Chevalier Jackson equipped the bronchoscope with an electric light source at the distal end and also added the suction channel.

Early in 1960, Japanese physician Shigeto Ikeda devised the means to replace the small electric bulb with glass fibers capable of transmitting brighter light from an outside source

He presented the first flexible bronchoscope at the International congress on diseases of the chest in Copenhagen (1996).

At the end of 1980s Asahi Pentax replaced the fibreoptic bundle with a charge coupled sensor at the tip of the scope. This video bronchoscope allowed the bronchoscopist to look at the monitor screen instead of through the eyepiece of the scope



Johnston and Frable (1845) stated that Exfoliative cytology was first used to study cells of the respiratory tract <sup>41</sup>.

Linder J. revealed its ability to diagnose malignancy as early as 1919 <sup>51</sup>, but it was not until 1950s and 1960s, that it was developed into a viable diagnostic modality.

In 1920, BAL was originally developed as a therapeutic procedure in the management of phosgene poisoning and also for treatment of pulmonary alveolar proteinosis and asthma.

In 1954, in the introduction of his famous Atlas of Exfoliative cytology, George N. Papanicolaou comments that the use of the cytologic method in the diagnosis of malignant lesions of the respiratory tract has been generally acclaimed as one of its most success applications.

Rigid bronchoscopic biopsy was the standard method of obtaining specimens for definitive diagnosis as a basis for management until the advent of flexible fiberoptic bronchoscopy in 1960s.

Dahlgren S. S. Nordenstron B stated that cytology was bolstered by the introduction of direct sampling methods via bronchoscopy and thoracic Fine Needle Aspiration (FNA) resulting in an armamentarium of sampling techniques.

In 1965 BAL was introduced initially as a therapeutic procedure to clear the alveolar spaces of accumulated

secretions blocking gaseous exchange, for example in alveolar proteinosis and bronchial asthma<sup>67</sup>.

Subsequently in 1984, the technique has been used for diagnostic purposes primarily in suspected pneumocystis carinii pneumonia, replacing open lung biopsy<sup>25</sup> and in 1987 in the diagnosis of interstitial lung disease (Stoller).

In 1985, it has been used to identify various other bacterial, fungal, parasitic, and sometimes viral agents causing pulmonary infections, particularly in patients with acquired immunodeficiency syndrome (AIDS)<sup>13, 46, 71</sup> and children with chronic granulomatous disease, an inherited defect of phagocytic oxidative enzymes.

In 1999 it has also been reported of value in investigating and monitoring other inflammatory reactions in the lung, for example ozone injury of the alveolar epithelium<sup>10</sup>, bronchiolitis obliterans organizing pneumonia (BOOP)<sup>47</sup> and chronic pulmonary diseases, mainly sarcoidosis and various forms of pulmonary pneumoconiosis.

In patients suspected radiologically of having pulmonary alveolar microlithiasis, a rare disease characterized by the presence of alveolar calcospherites, the calcospherites can be demonstrated in BAL fluid<sup>55</sup>.

BAL may sometimes disclose an unsuspected carcinoma particularly bronchioloalveolar carcinoma, which may mimic diffuse inflammatory lung disease radiologically and has been reported in patients monitored after lung transplantation <sup>28</sup>.

Quantization of lipid laden macrophages has been proposed as an index of aspiration pneumonitis <sup>20</sup>.

## EMBRYOLOGY

The respiratory system develops from the median diverticulum of foregut. The lining epithelium is therefore of endodermal origin. The connective tissue, cartilage and muscle, in relation to the organs of respiration are derived from splanchno – pleuric mesoderm.

Developmentally, the respiratory system is an outgrowth from the ventral wall of the foregut. The midline trachea develops two lateral outpocketings, the lung buds. The right lung bud eventually divides into three branches - the main bronchi - and the left into two main bronchi, thus giving rise to three lobes on the right and two on the left.

The lingula on the left is the middle lobe equivalent; however, the left lung is smaller than the right. The right main stem bronchus is more vertical and more directly in line with the trachea than the left.

The main right and left bronchi branch dichotomously, giving rise to progressively smaller airways. Accompanying the branching airways is the double arterial supply to the lungs that is the pulmonary and bronchial arteries.

During fetal life, all subdivisions of the bronchial tree are lined by cubical epithelium. With the onset of respiration after birth, the alveoli become dilated and the lining epithelium becomes thinned.

The pulmonary circulation is established early in fetal life. However most of the blood is short circuited through the foramen ovale and ductus arteriosus. The amount of blood circulating through the lungs progressively increases and by the seventh month of intrauterine life the circulation is rich enough to provide adequate oxygen for sustaining life. Hence an infant born thereafter is viable.

## **ANATOMY**

The respiratory tract can be categorized into upper and lower compartments. The upper airway extends from the sino-nasal area to the larynx. The lower respiratory tract which is the major focus of diagnostic respiratory cytopathology extends from the trachea to the lungs.

The tracheo bronchial tree divides progressively into smaller airways. Progressive branching of bronchi forms bronchioles which are distinguished from the bronchi by the lack of cartilage and sub mucosal glands within their walls.

Further branching of bronchioles leads to terminal bronchioles which are less than 2mm in diameter. The part of the lung distal to the terminal bronchiole is called the acinus. It is approximately spherical with diameter of about 7 mm.

An acinus is composed of respiratory bronchiole, which gives off several alveoli from their sides. These bronchioles then proceed into the alveolar ducts, which immediately branch into alveolar sacs.

## CELLULAR COMPONENTS OF THE RESPIRATORY TRACT

The trachea and bronchi are lined by pseudo stratified columnar epithelium. The predominant cell, the ciliated columnar cell, has a basal nucleus and finely granular chromatin.

At a ratio of approximately one per six ciliated cells, are goblet cells, with basally located nuclei and cytoplasm distended by mucus.

Adjacent to the basement membrane, are basal or reserve cells. They are small, undifferentiated cells that are presumed fore runners of the ciliated and goblet cells.

Neuroendocrine or (Feyrter or K Cells) kulchitsky cells are also present in the respiratory epithelium but cannot be identified with routine stains.

The terminal bronchioles are lined by predominantly non-ciliated cuboidal to columnar cells called Clara cells, usually not recognized on cytologic preparations.

The alveolar lining consists of type I and type II pneumocytes. Type I pneumocytes, which are more numerous, are paper thin, and cover the gas exchange portion of the alveolar surface.

Type II pneumocyte is plumper and more conspicuous, being cuboidal rather than flat. After lung injury, these cells function as reserve cells for the delicate type I pneumocyte.

Alveolar macrophages, loosely attached to the epithelial cells or lying free within the alveolar spaces, derived from blood monocyte and belonging to the mononuclear phagocytic system. Often they are filled with carbon particles and other phagocytosed materials.

## **GENERAL CYTOLOGICAL FINDINGS IN RESPIRATORY SAMPLES:**

### **Squamous cells**

Superficial and intermediate cells predominate in Pap stained smears. The intermediate cells are characterized by a round to oval vesicular nucleus, embedded in a uniform thin cyanophilic cytoplasm. The superficial cells have pyknotic nucleus and orangeophilic cytoplasm.

### **Bronchial Epithelial Cells**

Columnar or triangular in shape, the cells lie singly, in short ribbons or in flat sheets often have anatomical straight edge.

They have delicate cyanophilic cytoplasm. Nuclei vary considerably in size and shape, but are usually basal and rounded or oval, with open granular condensed chromatin and single small nucleoli. Multi nucleation may be seen.

Cilia are often still preserved, arising from a dark stained terminal bar at the broader end of the cell.

### **Goblet cells**

These are columnar cells but distended centrally by globules of mucin which overlies or displace the nucleus.

### **Reserve cells**

They form sheets of small regular cells slightly larger than lymphocytes with high nuclear cytoplasmic ratio, coarse chromatin and a narrow rim of cytoplasm.

### **Macrophages**

Round or oval dissociated cells. The cytoplasm is poorly defined, cyanophilic and often vacuolated, phagocytosed



material usually carbon may be present (Fig 1). They have central or eccentric nuclei, rounded or bean-shaped, with coarse chromatin and visible nucleoli.

Inflammatory cells, mainly polymorphonuclear leucocytes and lymphocytes, are invariably present in low numbers and are only of diagnostic significance if markedly increased.

Harmsen et al (1985) have shown that labeled particles instilled into the lung are not passively transported across the alveolar membrane but are phagocytosed by alveolar macrophages and then migrate to lymph nodes<sup>34</sup>.

Macrophages are the most numerous of cells recovered by lavage; phenomenal numbers are derived from the alveolar spaces in cigarette smokers.

In the normal non smoker, lymphocytes are the next most numerous cell followed by neutrophils and ciliated epithelial cells.

In cigarette smokers neutrophils are elevated in number as the result of airway irritation.

In both smokers and non smokers a small percentage of oropharyngeal derived squamous epithelial cells are normally present particularly in the bronchial specimen<sup>50</sup>.

## **NON CELLULAR INANIMATE COMPONENTS IN CYTOLOGY SPECIMEN:**

**Asbestos fibers** are composed of variable amounts of silica, magnesium and iron. In 1968, Gross P et al revealed that Ferruginous bodies were formed when filamentous dust particles such as asbestos becomes coated with protein and iron in the lung parenchyma<sup>31</sup>.

Gleich G (1977) stated in his book that **Charcot leyden crystals** were derived from the breakdown products of eosinophil granules, which would appear in conditions evoking pulmonary eosinophilia as yellow or pinkish stained needle crystals<sup>29</sup>.

Walker K. R. (1982) commented that coils of compressed mucus known as **Curshmann's spirals** were frequently seen in sputum from smokers or patient with obstructive airway disease, especially asthmatics. The spirals are casts of the small bronchioles and vary considerably in structure, formed from inspissated mucus<sup>2, 76</sup>.

Schmitz B & Pfitzer(1984) stated that **Psammoma bodies** (Calcospherites) are laminated non-refractile calcified concretions sometimes found in the presence of malignancy, although not necessarily closely associated with tumour cells<sup>72</sup>. Isolated psammoma bodies may be seen in the absence of any tumour formation.

In 1986, a study by Roggli et al of asbestos body counts in lavage fluid from 20 asbestos workers suggested that very low counts were non-specific and only high levels were indicative of significant industrial exposure.

The ferruginous bodies vary from 5-200µm in length; are light brown in colour and stain blue with Perl's stain for iron.

The bronchial washing specimens stained with Prussian blue to be more sensitive than sputum for the identification of asbestos bodies (Wheeler, TM, et al, 1988).

## **NON SPECIFIC REACTIVE CHANGES IN CYTOLOGICAL PREPARATIONS**

1956, Papanicolaou G.N. first observed **ciliocytophthoria**, a degenerative process affecting bronchial epithelium whereby columnar cells fragment into rounded cytoplasmic remnants, some of which still show tufts of cilia while other contain pyknotic nuclear material. This change was first observed in association with viral infections<sup>65</sup>. In 1961, Papanicolaou and his associates revealed that this change can be seen in a range of acute and chronic pulmonary diseases, and in cases of bronchial carcinoma<sup>64</sup>.

## **Squamous metaplasia**

Squamous metaplasia is one of the commonest responses of bronchial epithelium to persistent injury<sup>44, 59</sup> (Nasiell, M, 1965).

It is preceded by reserve cell hyperplasia and is a frequent finding in smokers<sup>4, 60, 70</sup>.

Reactive Squamous cells: They have slightly enlarged hyper chromatic nuclei and have come to be known as pap cells since first described by Dr. George Papanicolaou in his own sputum, due to laryngitis<sup>61</sup>.

**Hyperplasia of Bronchial Epithelium** is induced by many different noxious agents. In simple repair processes, sheets of actively regenerating cuboidal to columnar cells, with enlarged nuclei, fine chromatin and prominent nucleoli are seen.

**Reserve Cell Hyperplasia** is less easily recognized especially in sputum. Groups of small cohesive crowded cells with high nuclear / cytoplasmic ratio and dense chromatin are typical. Nuclear moulding may be present, hence confused with small cell carcinoma. Absence of necrosis and cell dissociation, uniformity of nuclear size and shape are helpful features<sup>56</sup>.

## **Hyperplasia of type II pneumocytes and bronchiolar cells**

The cells are polygonal or rectangular, occurring singly or in two or threes and may show cytoplasmic vacuolation. Nuclei are swollen with prominent nucleoli and pale or dense chromatin. They differ from hyperplastic bronchial cells in lacking cilia and are not associated with columnar or goblet cells nor with reserve cells.

## **CYTOLOGY OF RESPIRATORY INFECTIONS**

The role of the cytology laboratory in the diagnosis of opportunistic infection of the lower respiratory tract merits major considerations, because a number of these infectious agents readily lend them to detection and correct diagnosis by cytologic methods and principles.

### **Bacterial Pneumonia**

Sputum samples are commonest specimens. In 1981 Lazzari and associates explained that higher bacteria such as actinomyces organism have a more definitive appearance, forming colonies of radiating filamentous gram positive bacteria, which may be visible in microscopic samples as 'sulphur granules' <sup>48</sup>.

An analysis undertaken in Brazil by Tani et al in 1987 <sup>75</sup> of over 100 tuberculosis cytology samples other than FNAs revealed increased numbers of macrophages in 100%, excess neutrophils in 98% and increased lymphocytes in 85% of the

specimens. Epithelioid cells were present in 56% and giant cells in only 40% of the samples.

In 1989, Strigle S. M. and Gal A. A. explained that lavage fluid from cases of AIDS with tuberculosis typically contain many lymphocytes and enlarged foamy macrophages, but it is unusual to find a frankly granulomatous picture in these samples <sup>74</sup>.

In 1992, Chrell S.R. et al revealed that, the organism nocardia stains faintly pink by the Papanicolaou method, exhibits negative staining with MGG and is well demonstrated by Grocott's silver stain.

### **Viral infections**

In contrast to pneumonia due to bacteria, viral infection frequently induces specific cytopathic changes enabling the pathologist to give a firm indication of the causative agent.

Beale A.J. and Campbell W.A. (1959) explained multinucleation of epithelial cells as the characteristic feature of measles pneumonia, producing tightly clustered hyperchromatic nuclei at the center of the cell cytoplasm. Eosinophilic inclusion in both nucleus and cytoplasm may be seen in these multi nucleated cells <sup>8</sup>.

Naib Z. M. et al (1968) revealed that multi nuclear giant cells are the hallmark of Respiratory Syncytial Viral infection <sup>57</sup>.

Jain et al (1973) described basophilic inclusions in the cell cytoplasm during the course of illness <sup>38</sup>.

Warner et al <sup>77</sup> described the cytological findings in cytomegalovirus virus infection. Large inclusion bodies appear in the nuclei of macrophages and other cells of the respiratory tract in the course of infection. They are surrounded by a halo and the nuclear membrane is thickened giving an Owl's eye appearance. The number of inclusions is said to reflect the intensity of the infection <sup>1</sup>.

Sprigs A. I. et al (1982) stated that in viral infections clumps of hyper plastic epithelial cells may lead to a false diagnosis of adenocarcinoma.

Intranuclear inclusion bodies, loss of nuclear chromatin pattern, multi nucleation and cytoplasmic inclusion - The best recognized of these changes are seen in Herpes virus hominis infections (1983).

### **Fungal infections**

Infections and allergy are the two most important pathological effects produced in the lung by fungi. The presence of fungi in respiratory specimens raises one of three possibilities

First, they may be the contaminants from the mouth or atmosphere.

Secondly, there may be saprophytic colonization of an area of pre-existing diseased lung such as a cavity, without any invasion of tissues.

Thirdly, there could be an active infection (mycosis), with growth of fungi in the lung parenchyma.

*Blastomyces dermatidis*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Candida albicans*, *Paracoccidioides brasiliensis*, *Aspergillus* species are the few fungi which are identified in cytology specimens.

*Aspergillus* infection is a leading cause of death among immuno compromised patients. Cytologically, *Aspergillus* is a septate fungus having parallel walls with 45° angled branching.

Khan F. W. et al (1986), after a study of 82 immuno compromised patients undergoing bronchoscopic evaluation of new pulmonary infiltrates concluded that BAL sample was a rapid and effective technique for diagnosing invasive pulmonary Aspergillosis in the immuno compromised host.

Unfortunately, lavage had a low sensitivity for detecting *Aspergillus*, in the range of approximately 50%.



## **Parasitic Infections**

In 1985, Orenstein and associates reported a sensitivity of 94.4% using broncho alveolar lavage for the diagnosis of *Pneumocystis carinii* pneumonia in AIDS patients <sup>62</sup>.

## **NON - INFECTIVE GRANULOMATOUS LUNG DISEASE**

Infections such as tuberculosis are responsible for most of the treatable granulomatous diseases of lung but there are numerous other important disorders associated with granuloma formation, like sarcoidosis which is a systemic disease of unknown etiology affecting mainly young or middle aged patients, especially women. The respiratory tract is involved in over 90% of cases. Cellular immunity is depressed systemically, but there are increased numbers of activated T lymphocytes, especially T helper cells, at sites of granuloma formation <sup>37, 68</sup>.

Epithelioid histiocytes are the most characteristic feature, either dissociated or in clusters of spindle shaped cells with pale cytoplasm and elongated foot print shaped nuclei. Single cells are more often seen in sputum and washings.

Lymphocytes are found in abundance, especially in lavage fluid, reflecting profuse lymphocytic exudates into the alveoli while the disease is active. The lymphocytes are

predominantly of T cell type, constituting 10-70% of the inflammatory cell population, and are mainly T helper cells. Healthy non-smokers have levels below 7% <sup>43</sup>.

The ratio of lymphocytes to neutrophil polymorphs, macrophages and other inflammatory cells is helpful in supporting the diagnosis of sarcoidosis and in monitoring response to treatment or progression of the disease.

In the BAL specimens of patients with active tuberculosis and sarcoidosis, there is increased proportion of lymphocytes, predominantly activated T cells <sup>36</sup>. The CD-4/CD-8 ratio of lymphocytes was increased in sarcoidosis but not in tuberculosis.

A CD4/CD8 ratio  $\geq 2.5$  and the CD3/CD4 ratio  $\leq 0.31$  in broncho alveolar lavage lymphocytes are commonly seen in sarcoidosis<sup>45</sup>.

### HYPERSENSITIVITY PNEUMONITIS

Most commonly, hypersensitivity results from the inhalation of organic dust containing antigens made up of spores of thermophilic bacteria, true fungi, animal proteins, or bacterial products.

Farmer's lung results from exposure to dusts generated from harvested humid, warm hay that permits the rapid proliferation of the spores of thermophilic actinomycetes.

Pigeon breeder's lung (bird fancier's disease) is provoked by proteins from serum, excreta, or feathers of birds. Humidifier or air-conditioner lung is caused by thermophilic bacteria in heated water reservoirs.

Bronchoalveolar lavage specimens obtained during the acute phase show increased levels of proinflammatory chemokines such as MIP-1 $\alpha$  and IL-8.

Bronchoalveolar lavage specimens also consistently demonstrate increased number of T lymphocytes of both CD4 + and CD8 + phenotypes.

Histological changes in sub acute and chronic forms are characteristically centered on bronchioles. They include,

1. Interstitial pneumonitis consisting primarily of lymphocytes, plasma cells, and macrophages
2. Non caseating granulomas in two thirds of patients.
3. Interstitial fibrosis and obliterative bronchiolitis

In more than half the patients, there is also evidence of an intra-alveolar infiltrate.

### **Langerhans' cell Histiocytosis**

Langerhans' cell histiocytosis in the lung is part of spectrum of diseases characterized by monoclonal proliferation and infiltration of many organs by Langerhans' cells. The disease can occur as a single isolated nodule and mimic carcinoma of the lung.

When suspected clinically, the diagnosis can be supported by BAL in which more than 5% of large mononuclear cells are CD1a positive <sup>5, 19</sup>.

## INTERSTITIAL LUNG DISEASE AND PULMONARY FIBROSIS

Fibrosis of the lung develops either within alveolar spaces following active inflammation, resulting in fibrosing alveolitis or in the alveolar walls leading to interstitial fibrosis. In some patients both processes occur simultaneously.

The onset may be acute and severe as in the Hamman Rich syndrome, or insidious with gradual progression, the common course in idiopathic pulmonary fibrosis. An important subgroup includes patients with bronchiolitis obliterans in whom low grade inflammation and fibrosis affect the small airways.

In most cases cytology cannot determine the cause of the disease process but some indication as to prognosis and effectiveness of treatment can be obtained by monitoring inflammatory cell ratios in broncho alveolar lavage fluid.

There may be a predominant of neutrophil polymorphs, as exemplified by early idiopathic pulmonary fibrosis, occupational dust disease and collagen vascular disorders. These patients usually show an associated increase in macrophage and lymphocyte counts as well.

In the second group lymphocytes predominate, with virtually complete absence of polymorphs. Sarcoidosis and extrinsic allergic alveolitis are examples of this pattern. In sarcoidosis the lymphocytes are mainly of T helper subtype, whereas in extrinsic allergic alveolitis T suppressor cells predominate.

There may be marked eosinophilia in eosinophilic pneumonia and to a lesser extent in cryptogenic fibrosing alveolitis. The chest x ray shows diffuse infiltrates and bronchoalveolar lavage fluid contains more than 25% eosinophils. There is a prompt response to corticosteroids.

### **Diffuse Alveolar Damage**

Diffuse alveolar damage is the result of injury to distal alveoli from a single injurious event, usually within the prior few days to weeks. There are a great variety of causes including inhalants, drugs, oxygen toxicity, irradiation, shock, and sepsis.

Histologically there is an early, acute phase characterized by pulmonary edema, a proteinaceous exudate, and hyaline membrane formation. This is followed in a few days or a week by hyperplasia of type II pneumocytes in what is apparently a reparative effort. The damage may resolve or may be followed by organization and fibrosis.

Cytologic samples obtained by BAL early in the course of the disease consist of amorphous proteinaceous material,

alveolar macrophages, neutrophils, and atypical type II pneumocytes<sup>9</sup>. (Beskow et al, 2000)

### **Drug - Induced pulmonary toxicity**

Therapeutic agents may injure the lung directly by cytotoxic effects or a hypersensitivity reaction, or they may induce systemic conditions which in turn are associated with damage to the lung. A clear cut history of drug exposure is necessary for diagnosis.

Increased neutrophils are seen in lavage fluid in bleomycin induced toxicity, and are also a feature of other drug reactions (Ex: Gold therapy).

An unusual cytological picture has been documented with the anti arrhythmic drug amiodorone, which, in addition to causing damage to pneumocytes and pulmonary fibrosis, also produces changes in alveolar macrophages.

In lavage fluid the macrophages comprise up to 85% of the cell population and develop pale lacy cytoplasm due to the presence of numerous osmiophilic inclusion bodies, derived from damaged lysosomes.

### **Adult respiratory distress syndrome (ARDS)**

This serious form of lung damage was first described by Ashbaugh and associates in 1967 at which time it carried a mortality rate of nearly 70%.

The syndrome follows conditions such as severe trauma, pancreatitis or septicemia, developing within 1-3 days of these catastrophic illnesses, with the onset of acute pulmonary edema and inflammation accompanied by proliferation of type II pneumocytes and fibroblasts.

Local release of highly reactive free oxygen radicals and of enzymes such as proteases from neutrophils is postulated to cause direct damage to the lung parenchyma. Rapidly progressive pulmonary fibrosis may follow.

The cytological findings in adult respiratory distress syndrome have been recorded by Grotte et al (1990). He described exfoliation of clusters of hyper plastic broncho alveolar cells as seen in many other types of lung damage.

BAL has also been studied in adult respiratory distress syndrome (Hyers and fowler). This potentially lethal disorder is a complication of prolonged exposure to high concentration of oxygen. It is characterized by the presence of polymorpho nuclear leucocytes and high molecular weight plasma proteins in the lavage specimen, reflecting the increased permeability of damaged capillaries and interstitial tissues in alveolar septa.

### **Organ transplantation**

The lung is the seat of many of the complications arising in patients who have had organ transplantation, regardless of the nature of the transplant.

A recent and important application of BAL is detecting rejection and infection in recipients of lung transplants. Rejection is heralded by an increase in percentage of polymorphonuclear leucocytes in the lavage specimen <sup>18, 35</sup>.

### **Industrial exposure to chemicals and dusts**

Originally described in 1958 as farmer's lung, the underlying mechanism is a hypersensitivity pneumonitis, also known as extrinsic allergic alveolitis. It is an industrial hazard in a wide range of occupations in which there is exposure to moulds or animal proteins.

Lavage specimens show a raised lymphocyte count with an excess of T suppressor cells and sometimes of cytotoxic T cells.

Mineral pneumoconiosis may result from inhalation of dusts, gases or mineral fibers. Lavage fluid from cases of silicosis and other related pneumoconiosis shows raised levels of polymorphs initially and may also have increased numbers of lymphocytes and macrophages.

BAL specimens obtained in the acute phase show increased levels of proinflammatory chemokines such as MIP-1 $\alpha$  and IL-8.



BAL specimens also consistently demonstrate increased numbers of T lymphocytes of both CD4+ and CD8+ phenotypes.

### **Pulmonary Alveolar Proteinosis**

This rare disease was associated with known exposure to dusts in about half of the cases originally described by Rosen et al in 1958, but the condition is probably multifactorial in origin, and there is a strong association with immunosuppression. Lavage specimens are opaque or milky on gross inspection.

Rounded fragments of amorphous material of variable size, with amphophilic or eosinophilic staining properties are seen on light microscopy. Few if any inflammatory cells are seen.

Electron microscopy is necessary for a definitive diagnosis, revealing rounded lamellated structures identical to the osmiophilic bodies of type II pneumocytes and composed of surfactant.

### **Thermal injury**

A neutrophilic response in alveoli and airways is an early event. Healing is accompanied by squamous metaplasia. Reversible atypical squamous metaplasia has been observed in firemen.

A study by Clark et al of broncho alveolar lavage cells in 42 fire victims revealed that respiratory tract damage was greatest when smoke inhalation was accompanied by cutaneous burns.

Cytological findings include thick mucoid sputum or bronchial samples, many damaged epithelial cells, increased polymorphs and macrophages in lavage fluid. Squamous metaplasia occurs early. Secondary infection is common.

### **Role of Cytology in diagnosis of Non-Neoplastic pulmonary disease**

1. The first and most reliable is in confirmation of a diagnosis of infection by recognition of the causative agent or its cytopathic effects. Cytology provides a relatively cheap, quick, non-invasive and dependable method of diagnosis for these cases.

2. The next contribution lies in recognition of characteristic changes in respiratory tract samples which, when combined with clinical information, give direction or add weight to the clinical diagnosis.

3. Thirdly, negative findings in cytology are of value clinically since they contribute to the evidence required to exclude malignancy.

Table 1: BAL Cellular patterns as an adjunct to diagnosis<sup>21</sup>

Cellular	Diagnosis
Lymphocytic	Hypersensitivity pneumonitis Tuberculosis Sarcoidosis Berylliosis Malignant Infiltrates Drug induced pneumonitis HIV infection
Neutrophilic	Idiopathic pulmonary fibrosis Bacterial pneumonia Asbestosis Acute respiratory distress syndrome Wegener's Granulomatosis
Eosinophilic	Hyper eosinophilic syndrome Allergic broncho pulmonary aspergillosis Eosinophilic pneumonia Churg-Strauss syndrome
Mixed cellularity	Bronchiolitis obliterans organizing pneumonia (BOOP) Non specific interstitial pneumonia Inorganic dust disease

## **TUMOURS OF LUNG**

While respiratory cytodiagnosis had its birth in the late 1800s, rigid bronchoscopic biopsy was the standard method of obtaining specimens for definitive diagnosis as a basis for management until the advent of flexible fiberoptic bronchoscopy in the 1960s.

### **GENERAL APPROACH TO CYTOLOGICAL DIAGNOSIS OF NEOPLASIA**

The cytological appearance of the common lung malignancies vary in different types of specimen.

Samples taken by fiberoptic bronchoscopy are best examined together.

It is recommended that correlation of cytological and histological material should be done before final reports are issued.

Such correlation enhances sensitivity of diagnosis, optimizes tumour typing and may prevent confusion if material with different appearances is present in biopsy and cytological samples.

A 100% predictive value of malignant diagnosis is the aim, because cytological diagnosis is to be used for definite management decisions <sup>15, 16</sup>.

## **SQUAMOUS CELL CARCINOMA**

This form of lung carcinoma is usually located centrally within the lung in main bronchi or branches. The cytological diagnosis of squamous cell carcinoma from sputum samples or bronchial washings depends on the identification of abnormal squamous cells with malignant nuclear criteria, including enlargement, dense hyperchromasia, angularity and irregular chromatin distribution or 'black ink' chromatin.

### Sequence of epithelial events in the development of bronchogenic epidermoid carcinoma

Squamous metaplasia without atypia

Mild squamous dysplasia

Moderate squamous dysplasia

Severe squamous dysplasia

Carcinoma in situ (keratinizing and non keratinizing type)

Invasive carcinoma

### Cytopathologic Findings of squamous dysplasia

Dysplasia is cytologically graded based on nuclear morphology, amount of cytoplasm, and nuclear/cytoplasmic (N/C) ratios; the higher the ratio, the less differentiated the cell

is and the higher the grade of dysplasia. Cells are present isolated, in aggregates, and in tissue fragments.

Benign squamous metaplastic cells are round when present singly. When seen in tissue fragments, they show a honeycomb pattern with well-defined cell borders and small central nuclei with low nuclear to cytoplasmic ratio. Their cytoplasm is either cyanophilic or oxyphilic.

Mildly dysplastic cells are generally normal sized squamous metaplastic cells with slightly enlarged nuclei and granular chromatin with minimal increase in N/C ratio.

Moderately dysplastic cells may show variation in size with modestly enlarged nuclei containing coarsely granular chromatin. The cytoplasm is decreased in amount and may exhibit dyskeratosis.

Severely dysplastic cells tend to be very pleomorphic in size with variable cytoplasm that may or may not be dyskeratotic. The N/C ratios are likewise variable. Nuclear chromatin tends to be coarsely granular and deeply stained. Pyknosis is frequent. Nucleoli are not visualized.

As with any dysplastic lesion in the body, the diagnosis of pre neoplasia should be made with great caution in the presence of active chronic inflammation and also adult respiratory distress syndrome.

Similarly previous chemotherapy or radiotherapy may cause misdiagnosis. These latter two conditions should be suspected if there are large cells, with bizarre nuclei and plentiful cytoplasm

## SQUAMOUS CELL CARCINOMA

In 1935, a Finnish investigator, Lindberg, observed that squamous metaplasia is a frequent finding in the bronchi of patients with lung cancer.

Subsequent histologic studies, notably by Auerbah et al (1957) <sup>3</sup> supported this observation and squamous metaplasia was considered to be an important step in the genesis of bronchogenic squamous carcinoma.

This form of lung carcinoma is usually located centrally within the lung and present as hilar and perihilar masses. The tumors have a special tendency to undergo central necrosis with cavitations.

The cytological diagnosis of squamous cell carcinoma depends on the identification of abnormal squamous cells with malignant nuclear criteria including enlargement, dense hyperchromasia, angularity and irregular chromatin distribution of 'black ink' chromatin.

Histologically the tumor is characterized by the presence of keratinisation and intercellular bridges. Keratinisation takes the

form of squamous pearls or individual cells with markedly eosinophilic dense cytoplasm.

Morphologic variants of squamous cell carcinomas include:

1. Papillary or verrucous type,
2. Small cell variant,
3. Basaloid type,
4. Clear cell type, and
5. Spindle cell (sarcomatoid)/Carcino sarcoma

### **Adenocarcinoma**

Friedrich (1939), Rossle (1943), and later Spencer (1985) pointed out the association of peripheral lung cancers, mainly adenocarcinomas, with sub pleural scars<sup>27, 69</sup>.

Alveolar interstitial fibrosis is almost always present at the margins of scarring in the lung, regardless of cause, and is associated with reparative hyperplasia of bronchiolar and alveolar epithelial cells, a change that was thought to precede neoplasia in some cases.

The occurrence of bronchioloalveolar carcinoma in patients with scarring due to tuberculosis, cystic lung disease,



or long-standing scleroderma (progressive systemic sclerosis) of the lung are cited as examples of neoplasia arising in the epithelial hyperplasia that accompanies alveolar septal fibrosis<sup>63</sup>.

However, Shimosato et al (1982) have made a persuasive argument that most scars associated with lung cancer are formed after the tumor develops, and are caused by fibrosis in the areas of tumor necrosis.

Shimosato (1982) suggested that atypical adenomatous hyperplasia (AAH) of bronchoalveolar epithelium may arise in otherwise unremarkable lung tissue as a precursor of peripheral adenocarcinomas of the lung<sup>73</sup>.

Pneumocytes type II as well as Clara cells may participate in the pathogenesis of bronchioloalveolar carcinoma have been amply confirmed by histochemistry, electron microscopy, and immunopathology<sup>11, 54</sup>.

This is a malignant epithelial tumor with glandular differentiation or mucin production by the tumor cells. These lesions are usually more peripherally located and tend to be smaller. Peripheral adenocarcinomas are sometimes associated with areas of scarring. Cavitation is extremely unusual.

Adenocarcinoma appear in cytologic material as both single cells and cell clusters, cytoplasm homogenous to

extremely vacuolated, round to oval enlarged nuclei and finely granular chromatin. Centrally placed macro nucleoli is a prominent feature in adenocarcinomas of acinar type

Cell groups in specimens of adenocarcinoma consist of ball like clusters, papillary fragments, loose clusters or true acini with central lumina.

The two morphologic signs of glandular differentiation, often found together are formation of tubules or papillae and secretion of mucin.

The most recent (1999) World Health Organisation's (WHO) classification of lung tumours has proposed three major categories for adenocarcinomas:

#### Adenocarcinoma

- Acinar

- Papillary

- Bronchioloalveolar adenocarcinoma

  - Nonmucinous type

  - Mucinous type

- Solid adenocarcinoma with mucin production

#### Rare variants of adenocarcinoma

- Signet ring adenocarcinoma

- Mucinous carcinoma

- Adenocarcinoma with enteric and hepatoid differentiation

- Adenocarcinoma with choriocarcinomatous foci

## **Bronchioloalveolar Carcinoma**

BAC present as single peripheral nodule, multiple nodules and diffuse pneumonic- like infiltrate.

Histopathologically the mucinous type is formed by well differentiated mucin containing columnar cells that line respiratory spaces without invading the stroma. A sharp separation is often found between the neoplastic and normal cells, a useful diagnostic feature.

The cells of non mucinous type of BAC are cuboidal rather than columnar and have bright eosinophilic cytoplasm. Nuclear atypia and nucleolar prominence are greater than in mucinous variety. Hobnail cells and apical spouts may be present.

BAC tends to exfoliate both as single cells and cell groups. The nuclei are characteristically round to oval and uniform in size, with finely granular or powdery chromatin and small, inconspicuous nucleoli. A minority of cases, however, show prominent nucleoli. Nuclear folds are commonly present and in some cases nuclear pseudo inclusions are observed.

The cytoplasm varies in amount from modest to abundant and, like that of other adenocarcinomas, may be homogenous, granular, finely vacuolated, or distended by single or multiple large vacuoles.

## **Small Cell Carcinoma**

This tumour comprises 10 -20 % of all lung cancers. It is typically a lesion of central portion of lung.

Histopathologically the pattern of growth is generally solid but there may be streams, ribbons, rosettes and pseudo rosettes or tubules and ductules.

The classic form of small cell carcinoma is characterized by small round or oval cells resembling lymphocytes. The nuclei are finely granular and hyper chromatic, nucleoli are inconspicuous, mitoses are frequent and cytoplasm is so scanty.

Nuclear moulding a change first described in cytologic smears can also be appreciated in microscopic preparations.

Cytological findings are elongated groupings of small dissociating tumour cells, with scant cytoplasm, irregular moulded nuclei, coarsely stippled chromatin and inconspicuous nucleoli .Degenerative changes are common.

The WHO classification subdivides small cell carcinomas into those of oat cell type, where tumour cells are shrunken and more poorly preserved, tumours of intermediate type, with slightly more cytoplasm and larger better preserved nuclei, and combined carcinomas including areas of squamous

or adenocarcinoma; the latter constitute up to 10% of small cell tumours.

Bauer and Erozan reported psammoma bodies in a case of oat cell type of small cell carcinoma <sup>7</sup>.

### **Large Cell Undifferentiated Carcinoma**

Undifferentiated large cell carcinomas are pleomorphic malignant epithelial cells without definite evidence of either squamous or glandular differentiation. This tumour is composed of solid sheets of relatively uniform large tumour cells.

This neoplasm exfoliates large numbers of diagnostic cells that appear in respiratory specimens both as large single cells and syncytial groups. Cytoplasm usually cyanophilic and varies from granular to foamy with ill defined outlines.

Nuclei are round to lobulated with irregularly dispersed, intensely staining chromatin. Nucleoli may be large and vary in number from cell to cell. No evidence of keratinization is seen, and insufficient cytoplasmic differentiation is seen to warrant a diagnosis of adenocarcinoma.

#### **Variants of large cell undifferentiated carcinoma**

- Giant cell carcinoma

- Lymphoepithelioma like carcinoma

- Large cell neuroendocrine carcinoma and non-small cell carcinoma with neuro endocrine features

## **Safeguards against over diagnosis of malignancy**

In general, numerous clusters containing many cells are more suggestive of malignancy, whereas smaller groupings and single cells are more typical of alveolar cell hyperplasia.

The process of cilia around the periphery of cell clumps indicates a reactive rather than a neoplastic process.

## **SARCOMAS**

Primary pulmonary sarcomas are very uncommon and few are intra bronchial origin. Those that arise in the lung seldom erode the bronchial wall and they do not exfoliate easily.

The most common are leiomyosarcoma, malignant fibrous histiocytoma and fibro sarcoma, but precise classification of tumor type by cytology is difficult (Guiccon & Rossen) <sup>32</sup>. A specific classification requires histologic material and often a battery of immuno stains.

Further it should be emphasized that malignant spindle cells in sputum or a bronchial aspirate are much more likely to be derived from a spindle cell or sarcomatoid carcinoma.

It is quite possible for a sarcoma of lung to be misinterpreted as spindle cell carcinoma. Conversely a mistaken diagnosis of sarcoma may be due to exfoliated cells of

a primary or metastatic spindle cell carcinoma in lung (Nakajiima 1999)<sup>58</sup>.

### **Metastatic Carcinoma**

Metastatic tumours to the thorax constitute up to 15-20% of lesions diagnosed by cytology in some series<sup>42</sup>. Detailed knowledge of the clinical history must be available, together with earlier cytological and histopathological preparations for review and comparison with current material<sup>14, 40</sup>.

Metastatic tumour, particularly renal cell and colonic carcinomas<sup>12</sup>, may mimic primary tumours clinically either by growing as an endobronchial lesion or because of a bronchioloalveolar growth pattern.

Cytologically aggregates of tumour cells stand out in clean background. Cell blocks, special stains, immunocytochemistry helpful.

Some metastases are recognizable morphologically. For example, metastatic well differentiated colonic carcinoma often presents with pallisaded, elongated nuclei in aggregates and in a background of confluent necrosis<sup>26</sup>.

BAL was also very effective in the diagnosis of lymphangitic carcinomatosis caused by metastatic cancer<sup>49</sup>.

## **LYMPHOMA, LEUKAEMIA AND RELATED DISORDERS**

Non-Hodgkin's lymphomas of extra pulmonary origin and of all histological subtypes quite frequently affect the lung during the course of the disease.

Hodgkin's lymphoma is rarely seen as primary lung lesions<sup>79</sup>, but the lung is a common site of relapse, particularly for nodular sclerosing disease. Tumour deposits are usually nodular and may cavitate or produce endobronchial lesions.

Confirmation of recurrent tumour is often possible cytologically; on the other hand, definitive diagnosis of primary lymphoma generally requires open biopsy. Respiratory samples often provide a preliminary cytological diagnosis. However, sufficient material for immunophenotyping and / or genotyping is usually considered necessary for definitive diagnosis. It has been suggested that BAL samples may be useful for this purpose<sup>22</sup> and that immunoelectrophoresis of lavage fluid may also prove monoclonality<sup>30</sup>.

More readily diagnosed by BAL or FNA than in sputum. Large cell lymphomas are easier to diagnose than small / mixed. Loosely aggregated lymphoid cells, intact cytoplasm, vesicular nuclei, no moulding, and visible nucleoli are the features. Sub typing is possible in BAL and FNA material.



## **MATERIAL AND METHODS**

The present study was carried out in the Department of pathology, Madurai Medical College, Madurai, India for a period of 2 years from July 2006 to June 2008.

The cytological materials were obtained in the form of broncho alveolar lavage fluid using fiber optic bronchoscope. Specimens were collected from the Department of Thoracic Medicine, Government Rajaji Hospital, Madurai. During this 2 year study period 132 BAL samples were collected.

Details of the patients such as clinical history, personal history, and radiological investigations including details of bronchoscopy findings if any were recorded. The working proforma is appended in annexure I.

### Cytology:

The whole sample was taken in equal volumes in two clean glass test tubes and centrifuged for 5 minutes, at a speed of 2000 rpm. After centrifugation, the supernatant fluid was discarded and the sediments in the test tubes were pooled and smears were made on two clean grease free slides.

One of the smears should be air dried and stained with Giemsa's stain<sup>6</sup>. The other one should be kept in coplin jar containing isopropyl alcohol for 10-15 minutes and stained with Hematoxylin and eosin stain.

#### Histopathology:

The histopathology specimens of lung biopsy were fixed in 10% neutral buffered formalin. The tissues were processed, paraffin blocked, 5 microns thin sections were cut and stained with Hematoxylin and Eosin<sup>33</sup>.

Special stains such as PAS were used as and when required. Immuno histochemical studies<sup>39</sup> were done in relevant cases (Annexure III).

The results of histopathological study of H and E stained sections and cytological study of BAL were entered in the proforma. Photomicrographs of the smears and sections were taken whenever needed.

The information collected was recorded in a master chart. The results of both procedures were compared. The nature of study performed was cross sectional study. 'P' value analysis was used for statistical calculation to arrive at the conclusion.

Sensitivity, specificity and accuracy were calculated using the following formulae and taking HPE findings as the Gold standard.

Screening test results	Diagnosis	
	Diseased	Not Diseased
Positive	True positive TP	False positive FP
Negative	False negative FN	True negative TN

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}) \times 100$$

$$\text{Specificity} = \text{TN} / (\text{FP} + \text{TN}) \times 100$$

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{FP} + \text{FN} + \text{TN}) \times 100$$

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## **RESULTS**

In the present study, a total of 132 Broncho Alveolar samples were studied in the Department of Pathology, Madurai Medical College, Madurai during the period of July 2006 –June 2008

As per the proforma, the clinical data was collected from the patients who had undergone the BAL and biopsy procedures. After studying the BAL and biopsy samples, following observations were documented

In the present study, bronchoscopy was performed using flexible fiber optic bronchoscope followed by broncho alveolar lavage for 132 patients. The study was undertaken in patients presented with respiratory symptoms and radiologically visible pulmonary lesions.

Out of 132 BAL smears studied the diagnosis of malignancy was given in 36(27.2%) cases, dysplasia in 20 (15.2%) cases and 52(39.4%) cases were diagnosed as non neoplastic inflammatory lesions. The sample was inadequate to evaluate in 24 cases.

Histopathological correlation was available for 45 cases in which 30(66.7%) cases were diagnosed as malignant lesions, 5(11.1%) cases were dysplastic and 10(22.2%) cases were non neoplastic inflammatory lesions.

## **Sex Distribution**

Out of the total 132 BAL cases, 110 cases (83.3%) were males and 22 cases (16.7%) females (Table 3 and chart 1).M: F Ratio =5:1.

Out of the 45 biopsy samples studied 41 were males and 4 females.

## **Age incidence**

In the present study, the age group of patients ranged from 16 years to 80 years and the maximum numbers of cases were found in the age group of 51-60 years, comprising a total of 31.8% of the (42 cases) study population.

The least number of cases were in the age group of 71-80 years among males comprising only 0.75% of the study population (Table 4 and chart 2).

Among females, no cases were found in the age groups of 11-20 years, and 61-80 years.

The youngest patient among males was 16 year old and the oldest patient was 72 year old.

The youngest patient among females was 28 year old and the oldest patient was 60 year old.

### **Mean age of cases (Lesion based)**

In the present study the mean age for malignant lesions for males was 52.16 years, for females was 50.75 years and the mean age for both sexes was 52.00 years .

For dysplasia the mean age for males was 53.94 years for females was 46.75 years and the mean age for both sexes was 52.50 years

For inflammatory lesions the mean age for males was 51.93 years, for females were 39.7 years and the mean age for both sexes was 49.54 years (Table 5 and chart 3).

## **Smoking habit distribution**

Table 6 shows that significantly higher numbers of smokers (84 cases) were present among male population.

In total 63.6% of study population were smokers and 36.4 % were non- smokers. None of the female patients (22 cases) were smokers (chart 4).

## **Clinical symptoms**

Majority of the patients presented with clinical symptom (Table7 and chart 5) of cough followed by fever, breathlessness, hemoptysis and fever. Among the 132 cases cough was the presenting symptom in 126 cases (95.5%). Fever was one among the presenting symptoms in 64 cases (48.5%).

Breathlessness and hemoptysis were present in equal number of cases (41 cases-31.1%). Weight loss was seen in 30 cases (22.7%).

## **Cytological diagnosis in 132 cases**

Of the 132 cases, 108 cases had satisfactory smear. The diagnosis of malignancy was given in 36 cases, dysplasia in 20 cases, and 52 cases were diagnosed as non neoplastic inflammatory lesions. The sample was inadequate to evaluate in 24 cases (Table 8 and chart 6, 7).

In our study, majority of BAL samples showed features of inflammatory smear, comprising 52 cases, followed by 36 cases of malignancy and 20 cases of dysplastic lesions.

Among the 52 inflammatory cases, one known case of pulmonary tuberculosis on treatment showed chronic inflammatory smears and collections of epithelioid cells.

In the 36 malignant lesions diagnosed, 32 cases were males and 4 females. 16 males and 4 females were diagnosed as having dysplastic lesions. Among the 52 inflammatory lesions, 42 were males 10 were females.

During the study period 132 BAL samples were received for cytological examination. Out of these 132 samples, 45 cases had biopsy samples.



Out of the 45 smears studied, 11 cases(24.4%) were malignant lesions(Fig 9), 3cases(6.7%) were squamous cell carcinoma(Fig 4,5), 4 cases (11.1%)were adenocarcinoma (Fig 3),one case of undifferentiated carcinoma (Fig6,7,8),7 cases(15.6%) were dyaplastic and 15 cases (33.3%) were inflammatory smears (Fig 2). 4 cases had inadequate smears for evaluation.

### **Histopathological diagnosis**

The Histopathological diagnosis offered for the 45 cases is shown in table 9 (chart8, 9). Out of the 45 cases studied 30 cases (66.7%) were malignant lesions, 5 were dysplastic (11.1%) and 10 (22.2%) were non neoplastic lesions.

Among males, there were 28(68.3%) cases of malignant lesions, 5(12.3%) cases of dysplastic lesions and 8(19.5%) cases of inflammatory lesions.

Among females, there were equal number of cases of malignant (2 cases) and non neoplastic lesions (2 cases).

### **Distribution of malignant lesions in smokers**

Histopathological correlation was available for 45 cases in which 30 cases were diagnosed as malignant lesions.

Among the 30 cases, 22 cases were smokers and males. The remaining 6 males and 2 females were non smokers.

It is observed (Table 10 and chart 10) that the malignant lesions of the lung were more common in smokers than non smokers.

### **Distribution of malignant lesions on histopathology**

It is observed that there was no significant association between distribution of malignant lesion and sex of the patient. Out of the 30 cases of malignancy diagnosed in histopathology 28 were male and 2 were female. Majority of the cases were squamous cell carcinoma (Table 11 and chart 11).

Among males 11 cases were squamous cell carcinoma(Fig 18,19,20,21), 7 were adenocarcinoma (Fig 12), 3 cases of poorly differentiated adenocarcinoma (Fig 14,15,16,17), 2 cases of poorly differentiated carcinoma (Fig 13), 3 cases of anaplastic carcinoma(Fig 22,23,24), 1 case of bronchioloalveolar carcinoma and 1 case of small cell carcinoma (Fig 25) was diagnosed.

Among females, 1 case of bronchiolo alveolar carcinoma (Fig 10, 11) and 1 case of spindle cell sarcoma/? Sarcomatoid carcinoma (Fig 26) was reported.

Immunohistochemistry study of the spindle cell tumour was done. This tumour was immunoreactive for vimentin (Fig 28-weakly positive) and non reactive for markers such as cytokeratin, desmin and S-100 (Fig 27, 29, 30). Based on the marker study, the tumour was diagnosed as high grade fibro sarcoma.

### **Histopathological correlation of BAL cases**

Table 12 and 13 shows the histopathological correlation of BAL cases.

Out of the 30 cases of malignancy diagnosed histopathologically, the corresponding BAL smears showed malignant cells in 18 cases. In the remaining 12 cases of malignancy, BAL did not correlate with histopathological diagnosis. Out of 12 smears 6 showed features of inflammatory lesions, 3 were dysplastic and 3 cases were inadequate for evaluation.

Out of the 5 cases diagnosed as dysplasia, 3 cases of BAL did not correlate with histopathological diagnosis of dysplasia. Out of them, 1 case was inadequate for evaluation, 1 case showed features of malignancy and 1 showed features of inflammatory lesion.

Out of the 10 cases of inflammation diagnosed histopathologically, 8 cases of BAL showed inflammatory smears and 2 cases showed features of dysplasia.

### **Sensitivity and specificity**

#### **Malignant lesions:**

In the present study of 45 cases, the cytological diagnosis of malignancy was made in 19 cases, among them 18 cases were proved as malignancy in histopathology (True positive). One case was falsely diagnosed as malignancy in cytology and showed features of dysplasia in histopathology(False positive).Malignancy was diagnosed in 12 cases histopathologically but the corresponding cytology did not show malignant cells(False negative). Neither cytology nor histopathology showed malignancy in 14 cases (True negative).

True positive cases 18

False positive case 1

True negative cases 14

False negative cases 12

The sensitivity of BAL cytology in diagnosing malignant lesions in our study is 60% while the specificity is 93.3%. The diagnostic accuracy is 71.1%.

## **DISCUSSION**

Bronchoalveolar lavage can address many lesions that are diffuse or peripherally situated. The goal of BAL is investigation of pathologic conditions situated beyond the range of bronchoscopic visualisation.

Studies using BAL information for clinical correlation rest on the general concept that alveolar contents reflect parenchymal lung disease (86%).

The present study was conducted to analyse the cellular elements recovered from BAL in patients with pulmonary lesions. Based on the cytomorphology of the cells in the BAL, cytodiagnosis was provided. The BAL cytodiagnosis was correlated in patients who underwent bronchoscopic biopsy.

For the purpose of study, we have taken 132 cases of bronchoalveolar lavage samples, from the department of pathology, Government Rajaji Hospital, Madurai, during the period July 2006 – June 2008.

**Sex Incidence:**

Out of 132 cases 110 cases were males (83.3%) and 22(16.7%) cases were females. The male to female ratio is 5:1

**Age Incidence:**

In our study, the age of patients ranged between 16 years to 72 years. We received the samples from male cases from the age group of 11-80 years and the samples from female cases were from the age groups of 21-60 years.

It is observed that, the higher prevalence of respiratory diseases were in the age group of 51-60 years among males, and in the age group of 41-50 years among females.

The highest prevalence respiratory diseases among males and females were (62.8%) in the age group of 41-60 years.

**Smoking Habit:**

In the present study, out of 110 males 84 were smokers and 26 were non smokers. All 22 females were non smokers. It is observed that 76.4% of the study population was smokers and the habit of smoking is a major risk factor for pulmonary diseases.

**Clinical symptoms:**

It is observed that 126 cases (95.5%) suffered with cough followed by fever in 64 cases (48.5%), Breathlessness in 41 cases (31.1%), Hemoptysis in 41 cases (31.1%) and Weight loss in 30 cases (22.7%). So, cough being the most common clinical manifestation of patients with pulmonary diseases.

**Comparison of diagnostic accuracy for malignancy with other studies (Table 14):**

In 1987, Linder J et al studied BAL fluid among 35 cases of biopsy proven lung carcinomas. However 24 cases had cells diagnostic of malignancy on cytologic preparation of BAL fluid.

In 1992, Piruzynski M et al studied BAL fluid among 145 cases of biopsy proven lung carcinomas. Out of these 94 cases (64.8%) were found to have malignant disease of lung.

The prospective study by de Gracia J et al, in 1993 BAL were diagnostic in revealing malignant cells in 24 cases out of 55 cases with biopsy proven malignancy.

In a study by De beljek A et al in 1994, 61 patients were biopsy proven malignancies out of which 17 (27.9%) patients showed malignant cells in BAL fluid specimen.

In 1998, after studying 30 patients with lung cancers Wongsurakiat P et al, reported that BAL was positive for malignant cells in 14 patients (46.7%).

In the present study, 30 cases were diagnosed as malignant lesions in biopsy and corresponding BAL fluid samples examined show malignant cells in 18 cases (71.1%).

In the present study the diagnostic accuracy was 71.1% and had a near correlation with the study of Pirozynski M and Linder J et al.

Table 15 shows the comparison of histopathological diagnosis of lung lesions among BAL Cases with the study by Linder J et al 52. In the study by Linder J et al, the biopsy diagnosis of patients studied by BAL showed 10 cases of squamous cell carcinoma, 15 cases of adenocarcinoma, 7 cases of large cell undifferentiated carcinoma and 3 cases of small cell undifferentiated carcinoma.



In the present study, biopsy showed 11 cases of squamous cell carcinoma, 7 cases of adenocarcinoma, 2 cases of bronchioloalveolar carcinoma, 5 cases of poorly differentiated carcinoma, 3 cases of undifferentiated carcinoma, 1 case of small cell carcinoma and 1 case of spindle cell tumor (Table 15).

Immunohistochemistry study of spindle cell tumour showed reactivity for vimentin and the tumour is non reactive for cytokeratin, desmin and S- 100. Based on these observations the diagnosis of high grade fibro sarcoma was made.

Most of the patients with carcinoma in the study by Linder J et al were smokers and they had prominently neutrophils in the background of malignant cells.

In the present study also male patients diagnosed as having malignancy showed neutrophils predominantly in the background of malignant cells.

## **SUMMARY**

From the prospective study of BAL cytology done during the period of July 2006 – June 2008, the following features were observed

1. For the purpose of the study, 132 BAL sample has been presented.
2. Among the 132 cases, 110 cases (83.3%) were males, and 22 cases (16.7%) were females.
3. The youngest patient was 16 year old male, and oldest patient was 72 years male.
4. Among the 132 cases, 84 cases were smokers and all were males.
5. The remaining 26 males and all the 22 females were non-smokers.
6. Majority of the patients presented with clinical symptom of cough (95.5%) followed by fever (48.5%), breathlessness (31.1%), hemoptysis (31.1%) and weight loss (22.7%).
7. The maximum numbers of cases diagnosed cytologically were inflammatory lesions (39.4%).
8. Malignant, Dysplastic and Inflammatory lesions were more common among males (29%, 14.5% and 38.2%) respectively when compared to females (18.2%, 18.2% and 45.4%).
9. Histopathological correlation was available in 45 cases of which only 28 cases correlated.

10. Malignant lesions diagnosed histopathologically were more common in smokers.
11. The habit of smoking increases the risk of pulmonary cancer.
12. The diagnostic accuracy of BAL cytology in diagnosing malignant lesions was 71.1%.
13. Sensitivity and specificity for diagnosing malignant lesions by BAL cytology was 60% and 93.33% respectively.
14. The overall diagnostic accuracy of BAL was 71.1% in the present study.

## **CONCLUSION**

In conclusion a correlative study of broncho alveolar lavage cytology and histopathological examination of pulmonary lesions revealed the overall sensitivity of 60%, specificity of 93.33%, and accuracy of 71.1%.

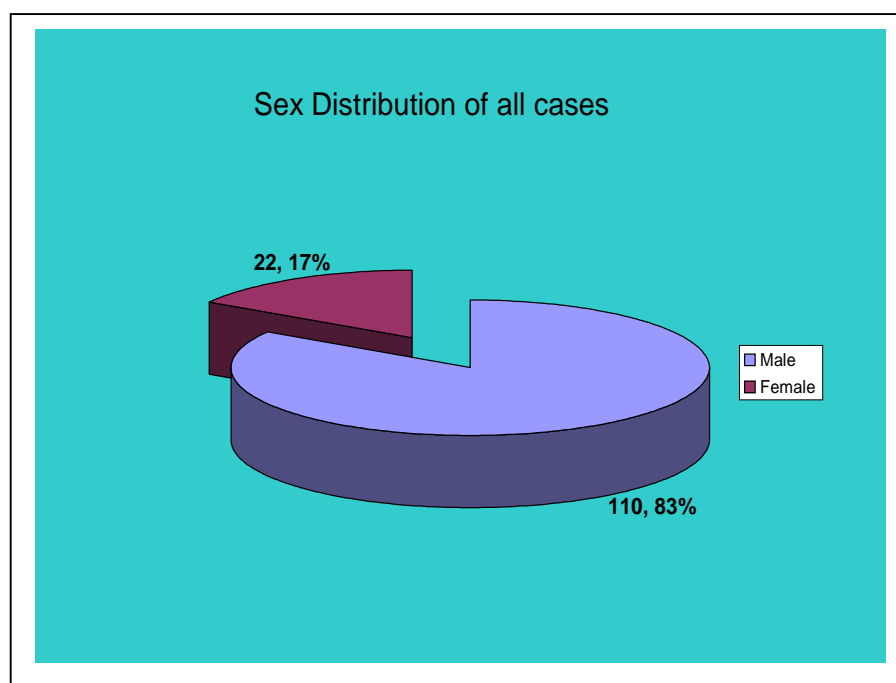
The false negative and false positive cases in this study can be minimized by proper sampling, screening, and strictly adhering to adequacy criteria.

The results are quite encouraging and BAL has a valuable role and is superior to other ancillary techniques of cytology in evaluating the pulmonary lesions, because of its safety, accuracy and minimal invasiveness.

**Table 3: Sex Distribution of all cases**

Sl.No.	Sex	No. of Cases	Percentage
1.	Male	110	83.3%
2.	Female	22	16.7%
Total		132	100%

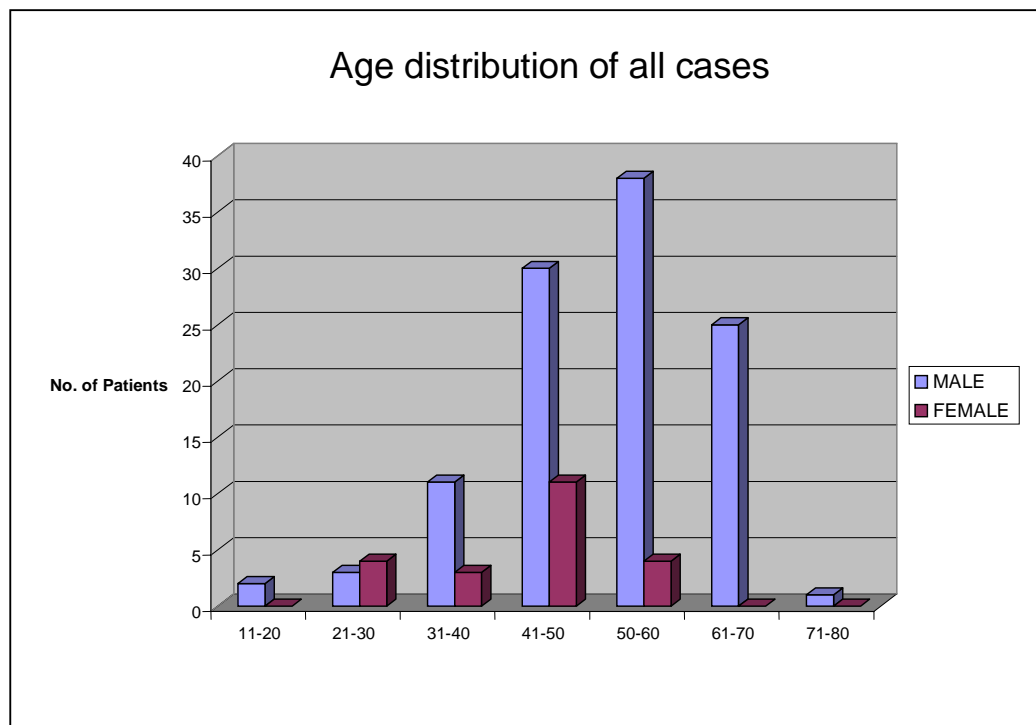
**Chart: 1**



**Table 4: Age Distribution of all cases**

Age Group (in years)	No. of Cases		Total
	Male	Female	
11-20	2 (1.8%)	-	2(1.5%)
21-30	3(2.7%)	4(18.2%)	7(5.3%)
31-40	11(10%)	3(13.6%)	14(10.6%)
41-50	30(27.2%)	11(50%)	41(31.0%)
51-60	38(34.5%)	4(18.2%)	42(31.8%)
61-70	25(22.7%)	-	25(18.9%)
71-80	1(0.9)	-	1(0.75%)
Total	110(99.8%)	22(100%)	132(99.9%)

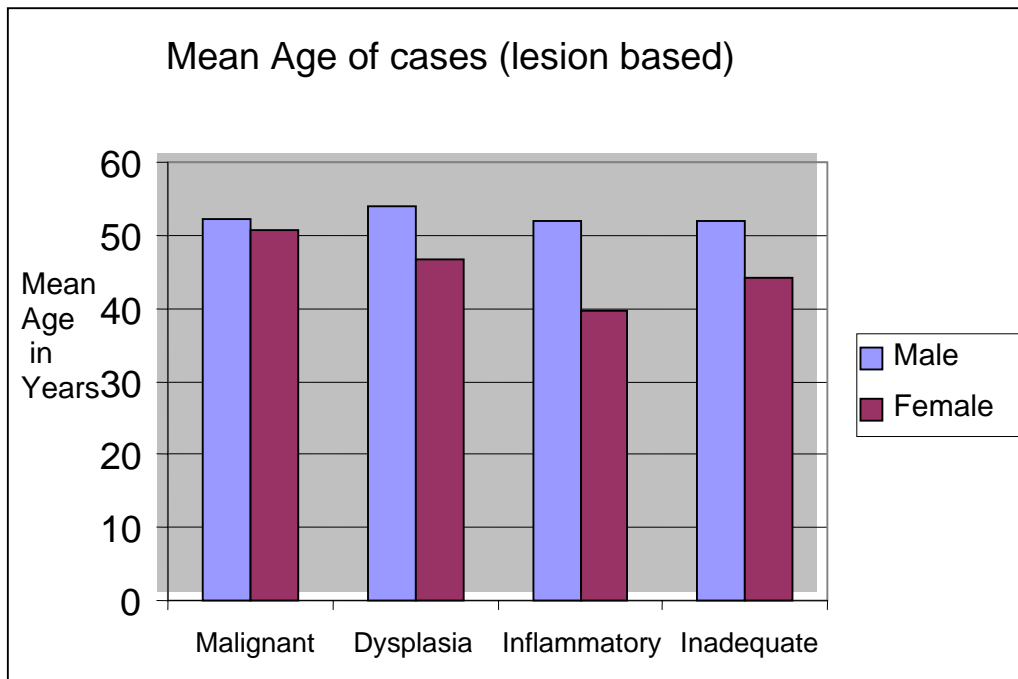
**Chart 2**



**Table 5: Mean age of cases (lesion based)**

	NO. OF CASES			MEAN		
	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL
MALIGNANCY	32	4	36	52.16	50.75	52.0
DYSPLASIA	16	4	20	53.94	46.75	52.50
INFLAMMATORY	42	10	52	51.93	39.7	49.54
INADEQUATE	20	4	24	51.90	44.25	50.63

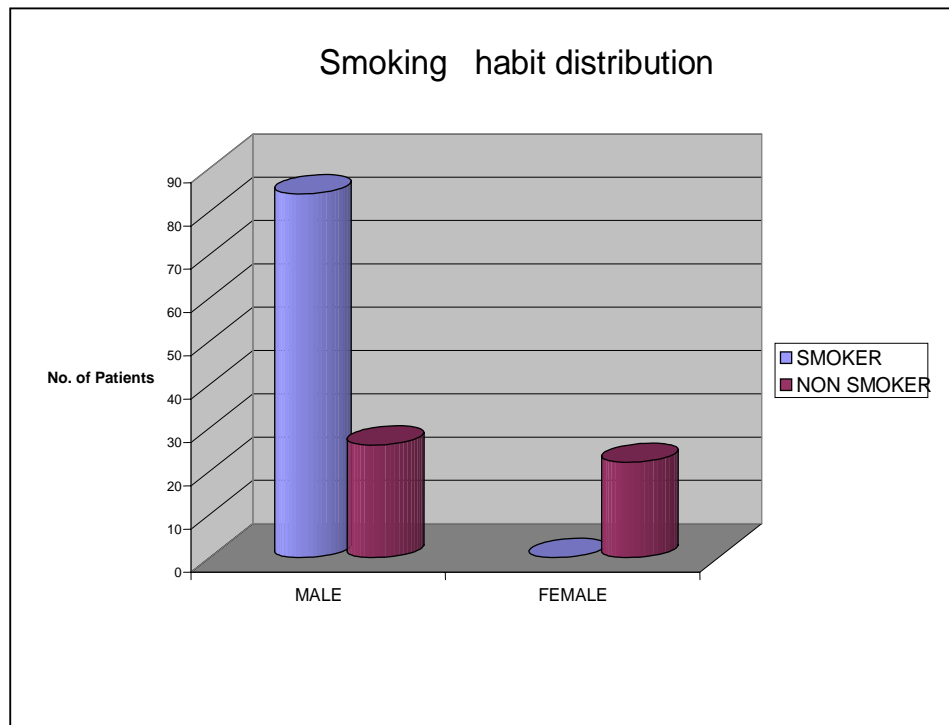
**Chart 3**



**Table 6: Smoking habit distribution of all cases**

Habit	Male	Female	Total
Smoker	84(76.4%)	-	84(63.6%)
Non-Smoker	26(23.6%)	22(100%)	48(36.4%)
Total	110(100%)	22(100%)	132(100%)

**Chart: 4**

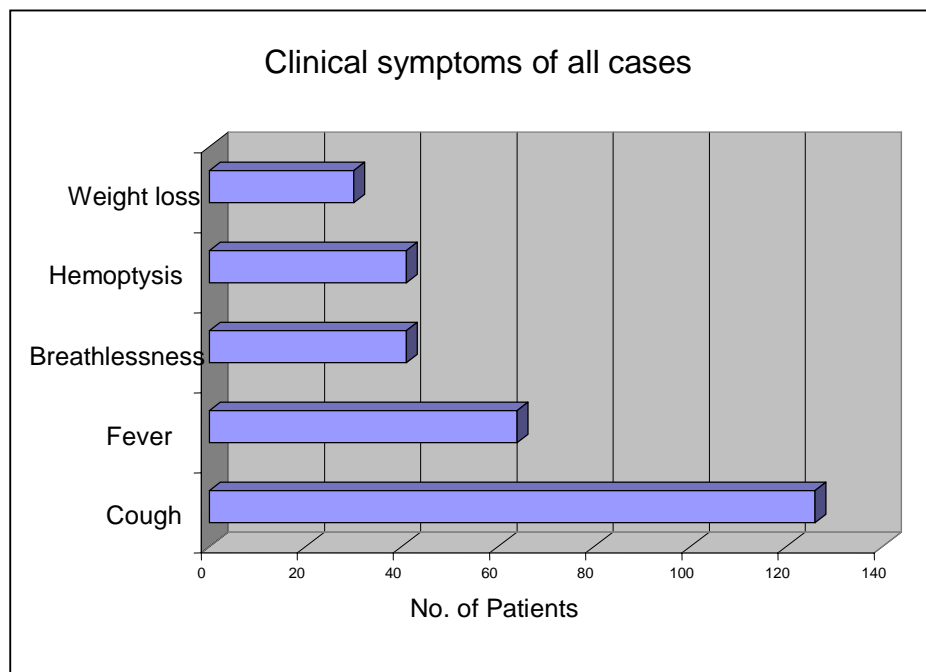




**Table 7: Clinical symptoms of all cases**

Sl. No	Clinical Symptoms	Found in	
		No. of cases	Percentage
1	Cough	126	95.5%
2	Fever	64	48.5%
3	Breathlessness	41	31.1%
4	Hemoptysis	41	31.1%
5	Weight loss	30	22.7%

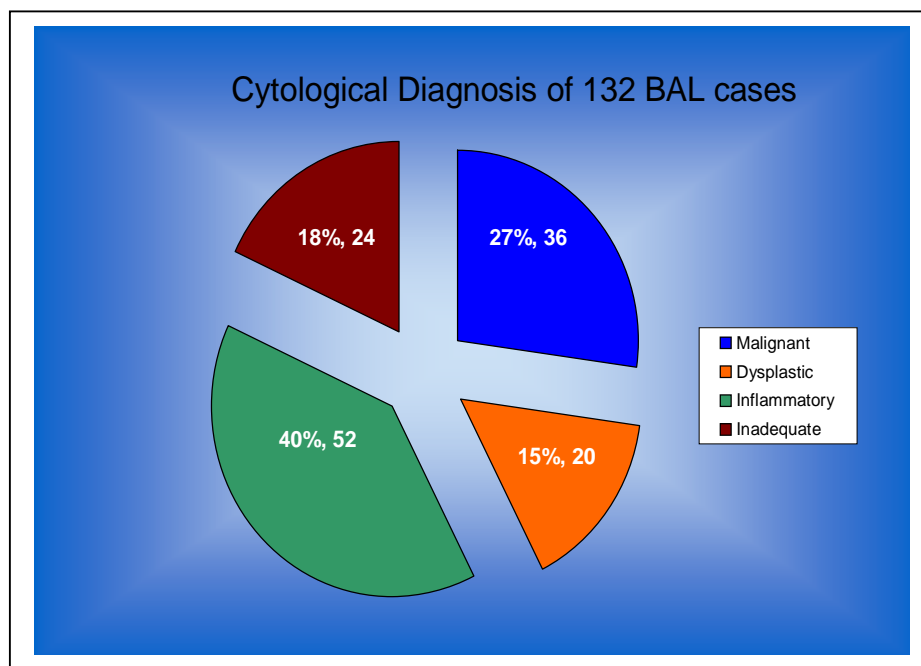
**Chart: 5**



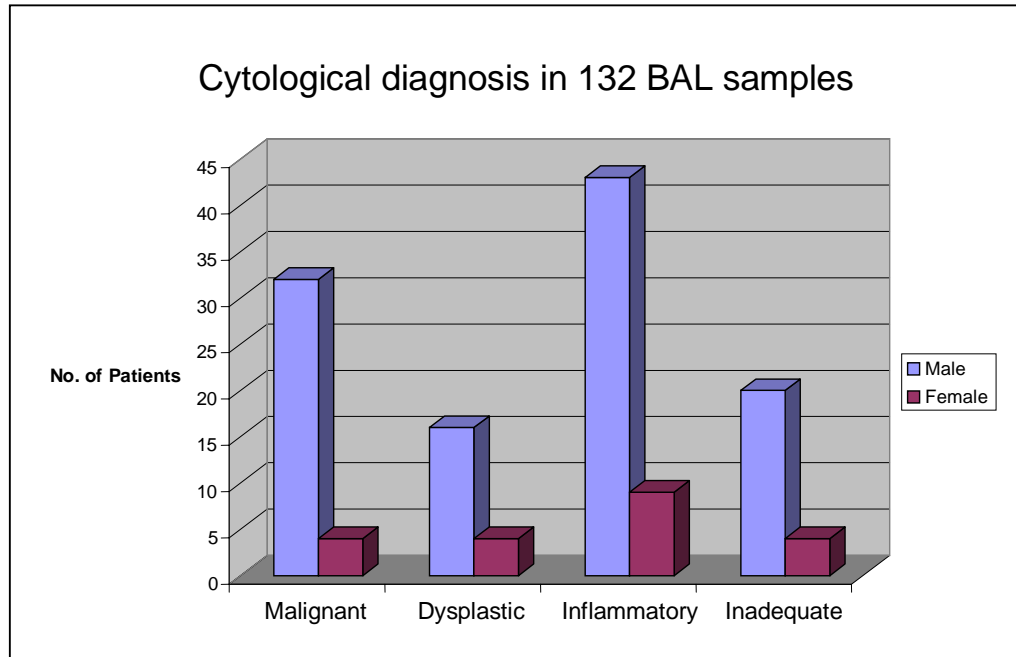
**Table 8: Cytological diagnosis in 132 BAL samples**

Sl. No	Lesion	No. of Cases		Total
		Male	Female	
1	Malignant	32(29%)	4(18.2%)	36(27.2%)
2	Dysplasia	16(14.5%)	4(18.2%)	20(15.2%)
3	Inflammatory	42(38.2%)	10(45.4%)	52(39.4%)
4	Inadequate	20(18.2%)	4(18.2%)	24(18.2%)
Total		110(100%)	22(100%)	132(100%)

**Chart: 6**



**Chart: 7**



**Table 9: Histopathological diagnosis of biopsy in 45 samples**

Sl. No	Lesion	No. of Cases		Total
		Male	Female	
1	Malignant	28(68.3%)	2(50%)	30(66.7%)
2	Dysplastic	5(12.3%)	-	5(11.1%)
3	Inflammatory	8(19.5%)	2(50%)	10(22.2%)
Total		41(100%)	4(100%)	45(100%)

Chart: 8

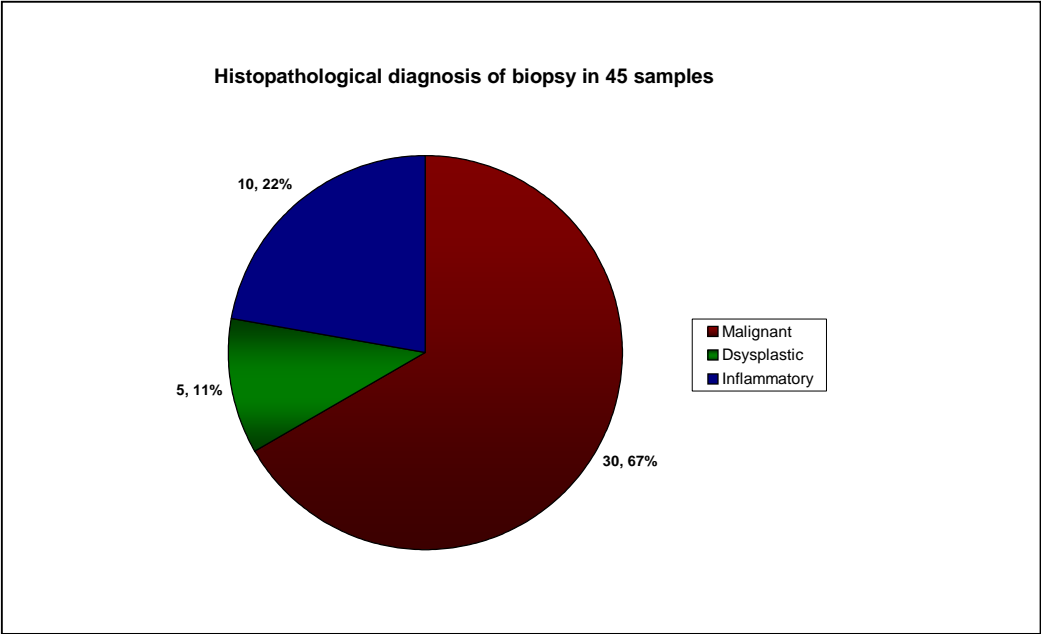
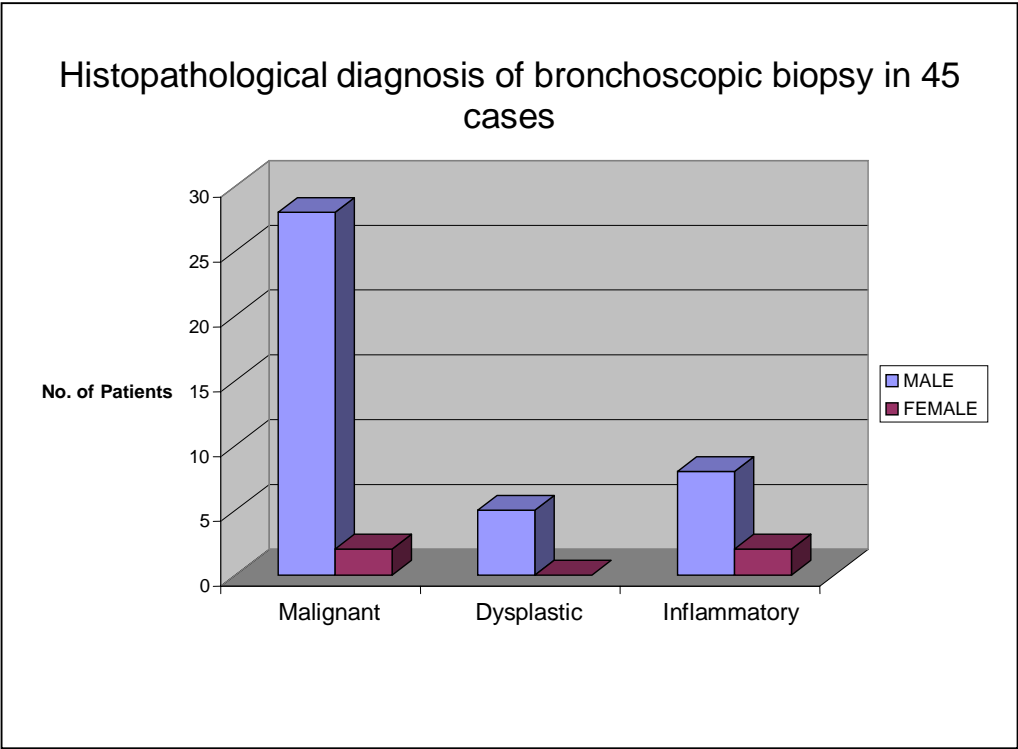


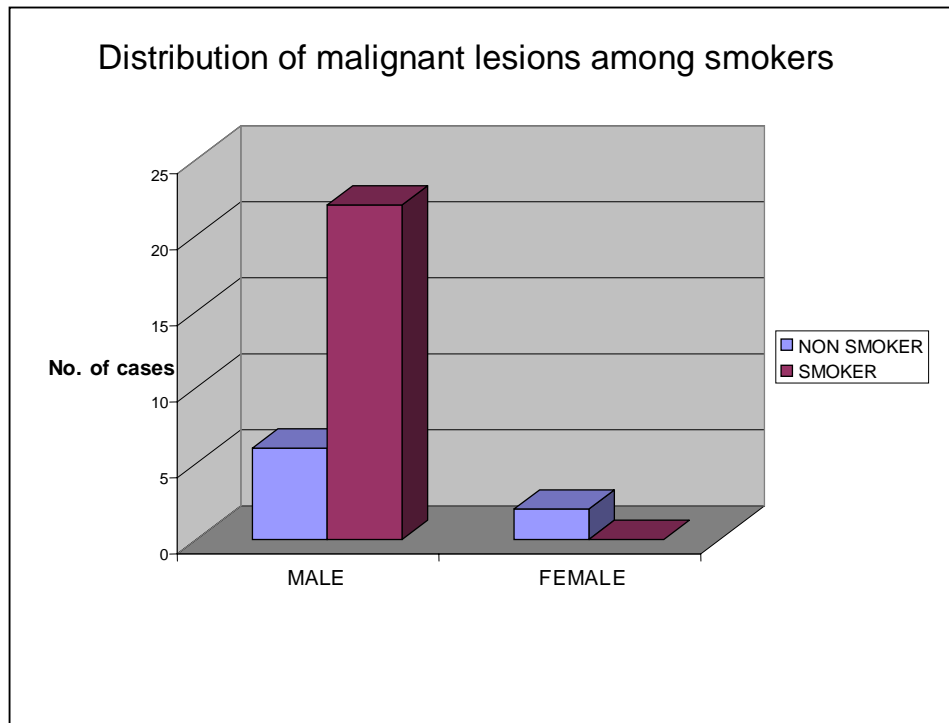
Chart: 9



**Table 10: Distribution of malignant lesions among smokers**

	Non-Smokers	Smokers	Total
<b>Male</b>	<b>6</b>	<b>22</b>	<b>28</b>
<b>Female</b>	<b>2</b>	<b>-</b>	<b>2</b>
<b>Total</b>	<b>8</b>	<b>22</b>	<b>30</b>

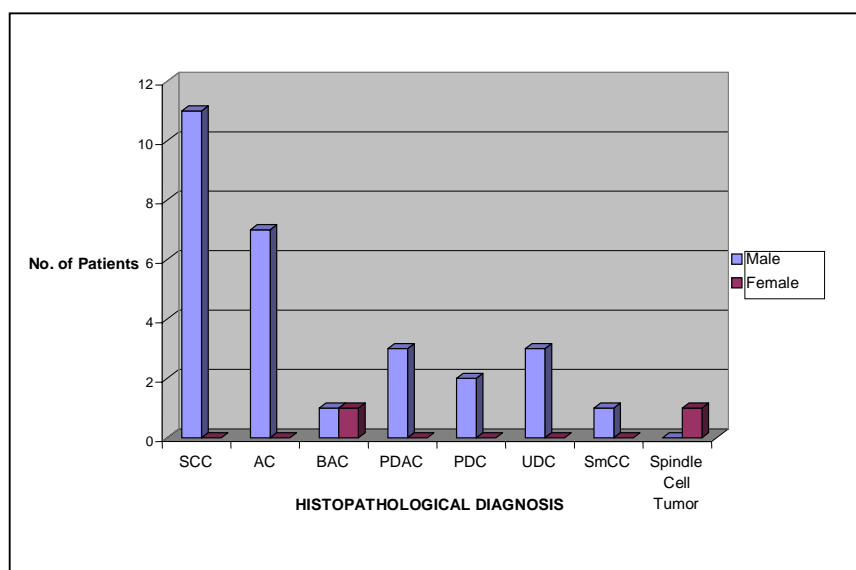
**Chart: 10**



**Table 11: Distribution of Malignant Lesions on Histopathology**

Sl. No	Lesion	No. of Cases		Total
		Male	Female	
1	Squamous Cell Carcinoma	11(39.3%)	-	11(%36.7)
2	Adeno Carcinoma	7(25)	-	7(23.3%)
3	Bronchiolo alveolar Carcinoma	1(3.6%)	1(50%)	2(6.7%)
4	Poorly differentiated adeno carcinoma	3(10.7%)	-	3(10.0%)
5	Poorly differentiated carcinoma	2(7.1%)	-	2(6.7%)
5	Anaplastic carcinoma	3(10.7%)	-	3(10.0%)
6	Small cell carcinoma	1(3.6%)	-	1(3.3%)
7	Spindle cell sarcoma /? Sarcomatoid carcinoma	-	1(50%)	1(3.3%)
<b>Total</b>		28(100%)	2(100%)	30(100%)

**Chart: 11**









**Table 12: Comparison of Cytological diagnosis of BAL with histopathological diagnosis**

Cytological Diagnosis	Final Histopathological Diagnosis																				
	NSI		DYS		SCC		AC		BAC		PDAC		PDC		UDC		SmCC		Spindle cell tumour		Total
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
INF	6	2	1		2		2		1		1										15
Malignancy			1		3		3			1			1		2						11
SCC					3																3
AC					1		1				2										4
UDC															1						1
DYS	2		2		1		1						1								7
IA			1		1												1			1	4
TOTAL	8	2	5	-	11	-	7	-	1	1	3	-	2	-	3	-	1	-	-	1	45

M – Male, F – Female, NSI – Non – Specific Inflammation, DYS – Dysplasia , SCC – Squamous cell carcinoma, AC – Adeno Carcinoma, BAC – Bronchiolo alveolar carcinoma, PDAC – Poorly Differentiated Adeno Carcinoma, PDC – Poorly Differentiated Carcinoma, UDC – Undifferentiated Carcinoma, INF – Inflammation, IA - Inadequate

**Table 13: Histopathological Correlation of BAL Cases**

<b>Lesions</b>	<b>Total</b>	<b>C</b>	<b>NC</b>
Malignant	30	18	12
Dysplastic	5	2	3
Inflammatory	10	8	2
Total	45	28	17

**Table 14: Comparison of diagnostic accuracy for malignancy with other studies**

Study	Total No. of proven malignancies	No. of cases diagnosed in BAL	Diagnostic accuracy (%)
Linder J et al <sup>52</sup>	35	24	68.6
Pirozynski M <sup>66</sup>	145	94	64.8
De Gracia J et al <sup>24</sup>	55	24	43.6
Debeljek A et al <sup>23</sup>	61	17	27.9
Wongsurakiat et al <sup>78</sup>	30	14	46.7
Present Study	30	18	71.1

**Table 15: Comparison of histopathological diagnosis of lung lesions among BAL Cases with the study by Linder J et al <sup>52</sup>**

Study	SCC	PDC	SmCC	ADC	BAC	Spindle cell tumour	Undifferentiated Carcinoma	
							Large Cell	Small Cell
Linder J et al 88	10 (29%)	-	-	15 (43%)	-	-	7 (20%)	3 (8%)
Present Study	11 (36.7%)	5 (16.7%)	1 (3.3%)	7 (23.3%)	2 (6.7%)	1 (3.3%)	3 (10%)	-

**Table 2: Diagnostic BAL findings <sup>21</sup>**

Sl.No.	BAL findings	Diagnosis
1	Pneumocystis carinii, fungi, cytomegalovirus transformed cells	Opportunistic infections
2	Milky effluent, periodic acid Schiff positive non cellular corpuscles, amorphous debris, foamy macrophages	Alveolar proteinosis
3	Hemosiderin laden macrophages, intra cytoplasmic fragments or red blood cells in macrophages, free red blood cells	Alveolar hemorrhage syndrome
4	Malignant cells of solid tumors, lymphoma, leukemia	Malignant infiltrates
5	Dust particles in macrophages, quantifying asbestos bodies	Dust exposure
6	Eosinophils greater than 25%	Eosinophilic lung disease
7	Positive lymphocyte transformation test to beryllium	Chronic beryllium disease
8	CD1 positive Langerhan's cells increased	Langerhans cell histiocytosis
9	Atypical hyper plastic type II Pneumocytes	Diffuse alveolar damage, drug toxicity

**ANNEXURE – I**

**PROFORMA**

**BRANCHO ALVEOLAR LAVAGE CYTOLOGY IN  
PULMONARY LESIONS**

1. Name : Age: Sex:  
2. Address : Unit: Ward:

IP / OP No.:

Cytology No.:

**3. Presenting Complaints**

- a. Fever                      b. Cough
- c. Expectoration      d. Hemoptysis
- e. Chest Pain              f. Breathlessness
- g. Loss of appetite              h. Weight loss

**4. Past History:**

**5. Treatment History:**

6. Personal History:              Smoker  
Non-Smoker

**7. Occupational History:**

**8. Family History:**

## 9. Investigations:

### A. Hematological

## B. Biochemical

### C. Radiological Diagnosis

X-ray chest

CT scan

D. Fiber optic Bronchoscopic findings:

### E. Others

## 10. Clinical Diagnosis:

## 11. BAL Cytology:

Specimen : Adequate / Inadequate

Squamous Cell : Metaplastic / Dysplastic / Malignant

Columnar Cell : Reactive / Malignant

Inflammatory Cells: Neutrophils / Eosinophils /  
Lymphocytes / Macrophages

Non-cellular material:

Background :

Organism : Fungi

12. Histopathological Correlation if any:

13. Special study if any:

14. Final Diagnosis:

## **ANNEXURE - II**

### **ELECTIVE BRONCHOSCOPY AND BAL**

#### Patient Preparation

1. Fasting before the procedure to reduce the risk of aspiration (> 8 hours preferable)
2. Informed consent
3. Optional premedication (e.g. 0.5 mg atropine, IM to reduce secretions)
4. Topical anesthesia (e.g. 0.45% tetracaine)

#### Routine monitoring during bronchoscopy

Continuous pulse oximetry  
Blood pressure  
Heart rate and  
A continuous electro cardiogram

All patients should receive supplemental oxygen during and after the procedure.

The flexible bronchoscope can be inserted either trans nasally or transorally. We prefer trans nasal approach, because it is simple. When using the trans nasal approach, the nasal fossa and nasopharynx should be carefully examined before the flexible bronchoscope is passed into the larynx.

Once the larynx is reached, advancement of the bronchoscope is halted. At this point the vocal cords can be anesthetized, examined for normal abduction and adduction and assessed for pathology (polyps, lesions, and erythema). Next the flexible bronchoscope is passed through the cords and the circumference of the trachea is carefully examined for mucosal changes and lesions, external compressions and abnormalities of the cartilaginous rings.

Then the carina is examined for splaying, position and mucosal abnormalities. All segmental bronchial orifices are inspected systematically with careful attention paid to their color, texture, position, relative size, patency and the presence of splaying. The bronchial mucosa should be assessed carefully to identify sub mucosal infiltration, degree of acute or chronic inflammation and nature and quantity of secretion.

A variety of sampling techniques, including bronchial washing, bronchial brushing, BAL, Endobronchial or transbronchial forceps biopsy and endobronchial or trans bronchial needle aspiration, allows the operator to collect specimens from the respiratory tract.

The goal of BAL is investigation of pathological conditions situated beyond the range of bronchoscopic visualization.

The bronchoscope is physically wedged into the bronchus. Warmed saline is then used to flood the bronchial



and alveolar tissue distal to this point. Up to one million alveoli may thus be sampled.

Fluid is introduced in aliquots that may range from 20 to 100 ml or more. After each fluid instillation, the bronchus is aspirated, and in most instances, up to 50% of the initial fluid volume is recovered.

Differential washings are an accepted part of the procedure, the first to remove bronchial luminal content and the second to provide a good alveolar sample<sup>53</sup>.

Up to 30% of routinely submitted samples may have a paucity of macrophages, an excess of bronchial epithelial cells, a mucopurulent component or degenerative changes rendering them unsuitable for assessment<sup>17</sup>.

A series of four or five separate instillations follows in rapid succession. The returned fluid can be used for cytological examination and enumeration with differential counting of inflammatory cells and in microbiologic studies.

The first aliquot is often heavily contaminated with upper aero digestive tract material, the presence of which makes it difficult to evaluate pulmonary parenchymal disorders.

Thus excluding this first sample, pooling the remaining three of four aliquots for the studies is suggested by many investigators.

The aspirated bronchio alveolar lavage sample sent immediately to the laboratories in sterile labeled containers without adding any fixatives.

The samples were accompanied by appropriately filled requisition form. After receiving the BAL fluid sample in the laboratory, it was verified with the request form.

Then the whole sample was taken in equal volumes in two clean glass test tubes and centrifuged for 5 minutes, at a speed of 2000 rpm.

After centrifugation, the supernatant fluid was discarded and the sediments in all the test tubes were pooled and smears were made on two clean grease free slides.

One of the smears should be air dried and stained with Giemsa's stain. The other one should be kept in coplin jar containing isopropyl alcohol for 10-15 minutes and stained with Hematoxylin and eosin stain.

**Broncho alveolar lavage specimen adequacy:**

Assessment based primarily on cell counts.

At least  $2 \times 10^6$  total cells obtained.

More than ten macrophages per high power microscopic field(Fig 1).

More than 25 macrophages per high power field in the presence of upper tract contamination or acute inflammatory exudates.

## **Unsatisfactory:**

Based primarily on other criteria

Excessive squamous or bronchial cells (epithelial cells > macrophages).

Excessive degeneration.

Excessive red blood cells, especially if other adequacy problems exist.

## **Factors affecting the cellular yield of Broncho alveolar lavage in Healthy controls.**

### Patient Factors:

Smoking without chronic bronchitis yields more macrophages

Smoking with chronic bronchitis yields more neutrophils.

### Procedural factors:

Lung area studied

Volume instilled (aliquot size and number of aliquots).

### Preparatory factors:

Separate processing of the first (bronchial) aliquot

Gauze filtration to remove mucus and bronchial cells.

Cytocentrifugation causes selective loss of lymphocytes.

Membrane filtration causes selective loss of neutrophils.

Bronchoalveolar lavage is used with a high degree of success both for diagnostic and therapeutic purposes.

### **Diagnostic Application of BAL**

1. For the detection of opportunistic infections in Immunocompromised hosts
2. In the detection of lung cancers
3. Evaluation of interstitial lung diseases
4. Pneumonia in immunocompromised patients to rule out an infectious process and to identify the offending microorganisms.
5. Persistent and unexplained pulmonary infiltrate in both immunocompromised and immunocompetent patients.
6. In patients with idiopathic pulmonary hemosiderosis, pulmonary interstitial disease, and drug toxicity.
7. To monitor drug response by performing differential count.
8. In post transplant patients, to monitor the progress, identify acute and chronic rejection and infections.
9. Useful in identification of lipid-filled histiocytes in aspirations pneumonias.
10. To confirm fat embolization from infarcted bone marrow in acute chest syndrome in sickle cell disease.
11. Identify leukemic infiltrate.
12. To confirm histiocytosis X, by identifying Langerhan cells.

**ANNEXURE - III**  
**MAY– GRUNWALD – GIEMSA TECHNIQUE <sup>6</sup>**

Stock stain            -    Giemsa powder 0.38 gm  
                                      Glycerin 25 ml  
                                      Methyl alcohol 25 ml

Working solution - To 1 ml of stock solution add 10 ml  
of distilled water

**STAIN PROCEDURE**

Immerse air dried BAL smears in May – Grunwald  
working stain for 15 minutes. Rinse gently in tap water.  
Immerse smears in Giemsa stain for 15 minutes. Rinse gently in  
tap water. Allow to air dry. Dip in xylene and mount in DPX.

## **IMMUNO HISTOCHEMICAL STAINING <sup>39</sup>**

- 1.** Sections to alcohol
- 2.** Block endogenous peroxidase activity by incubating in hydrogen peroxide solution for 30 minute.
- 3.** Hydrate sections by passing through graded ethanol series and wash in running water for 15 minutes.
- 4.** Incubate sections in the normal swine serum diluted in Tris / saline for 15 minutes.
- 5.** Drain off excess Tris buffered normal swine serum.
- 6.** Incubate sections with primary antiserum diluted 1; 2000, 1:1000, 1:250 and 1:100 in 1% normal swine serum for 30 minutes.
- 7.** Jet wash off excess antiserum and then wash slides in Tris/ saline for three 2 minutes changes.
- 8.** Incubate sections in swine anti rabbit IgG diluted 1:20 for 30 minutes.
- 9.** Jet wash of excess antiserum and then wash slides for three 2 minutes changes.
- 10.** Incubate sections in PAP complex diluted 1: 60 in 1% normal swine serum in Tris / saline for 30 minutes.
- 11.** Jet wash off excess complex and wash in Tris / saline for three 2 minutes changes.
- 12.** Incubate sections in DAB medium for 5 minutes.
- 13.** Wash sections in running water for 10 minutes
- 14.** Counterstain in alum haematoxylin, dehydrate clear
- 15.** and mount.

Results : Reaction product ..... Brown.

Nuclei ..... Blue

## **PERIODIC ACID SCHIFF TECHNIQUE**

- 1.** Bring sections to water
- 2.** Oxidize for 5-10 minutes in 1% aqueous periodic acid.
- 3.** Wash in running water for 5 minutes and rinse in distilled water
- 4.** Treat with Schiff reagent for 10-30 minutes
- 5.** Transfer to directly to first sulphite rinse for 1 minute and to the second sulphite rinse for 2 minutes. Transfer to the third sulphite rinse for 2 minutes.
- 6.** Wash for 10 minutes in running water.
- 7.** Counter stain with haematoxylin.
- 8.** Dehydrate, clean and mount in D.P.X.

**RESULT:**

PAS positive substances are bright red in colour

**ANNEXURE - IV**  
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## CODES

M-Male

F-Female

S-Smoker

NS-Non smoker

Dys-Dysplasia

INF-Inflammatory

IA-Inadequate

A - Squamous cell carcinoma

B - Adenocarcinoma

C - Bronchioloalveolar carcinoma

D - Poorly differentiated adenocarcinoma

E - Poorly differentiated carcinoma

F - Anaplastic carcinoma

G - Small cell carcinoma

H - Spindle cell sarcoma / Sarcomatoid carcinoma